SEQUENCE ANALYSIS AND EXPRESSION OF ARGININE KINASE mRNA IN GILLS OF THE SEMI-TERRESTRIAL GRAPSID CRAB *PACHYGRAPSUS MARMORATUS*

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The grapsid crab *Pachygrapsus marmoratus* is a strong hyper/hypoosmoregulator, maintaining its hemolymph osmolality at relatively constant levels in a broad range of environmental salinities (Pierrot et al., *Arch. Physiol. Biochem.* 103: 401-409, 1995). Osmoregulation in euryhaline crabs is thought to be accomplished primarily by active NaCl transport across posterior gills, while anterior gills are specialized for gas exchange and other functions (reviewed in Pequeux, *J. Crust. Biol.*, 15: 1-60, 1995). The active uptake and excretion of ions by ion transporters depend on a ready supply of metabolic energy. In the present study, we asked whether gills of *P. marmoratus* express one likely component of the energetic pathway, namely arginine kinase. Arginine kinase catalyzes the reversible dephosphorylation of phosphoarginine, contributing to the restoration of adenosine triphosphate concentrations in cells experiencing high and variable demands on their reserves of high-energy phosphates.

Crabs were caught in rocky areas of the Mediterranean coast nearby Montpellier, France. They were maintained in circuits of individual boxes supplied with aerated and filtered seawater until their first molt. In the intermolt stage, they were acclimated to three different salinities of 10‰ (D), 37‰ (S) and 45‰ (C) for 8 to 10 days. Crabs were then dissected and gills were cut off at their base and preserved in RNA*later* (Ambion) to bring them to MDIBL.

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1 PACHYGRAPSUS MADAATISKLEEGFKKLQGATDCKSLLKKYLTKDVFDQLKAKKTSLGATL 50
2 CARCINUS     MADAATITKLEEGFKKLEAATDCKSLLKKYLTKSVFDQLKAKKTSLGATL
3 LIMULUS      MVDQATLDKLEAGFKKLQEASDCKSLLKKHLTKDVFDSIKNKKTGMGATL
4 APIS         MVDQAVLDKLETGFSKL-SSSDSKSLLKKYLSKDVFDQLKTKKTSFDSTL
5 Consensus    M D A   KLE GF KL    D KSLLKK L K VFD  K KKT    TL

1 PACHYGRAPSUS LDVIQSGVENLDSGVGVYAPDAEAYTLFAPLFDPIIEDYHKGFKQTDKHP 100
2 CARCINUS     LDVIQSGVENLDSGVGVYAPDAEAYTLFSPLFDPIIEDYHKGFKQTDKHP
3 LIMULUS      LDVIQSGVENLDSGVGIYAPDAESYRTFGPLFDPIIDDYHGGFKLTDKHP
4 APIS         LDCIQSGIENLDSGVGIYAPDAEAYTLFADLFDPIIEDYHGGFKKTDKHP
5 Consensus    LD IQSG ENLDSGVG YAPDAE Y  F  LFDPII DYH GFK TDKHP

1 PACHYGRAPSUS NKDFGDVNQFVNVDPDGKFVISTRVRCGRSMEGYPFNPCLTEAQYKEMEA 150
2 CARCINUS     NKDFGDVNQFVNVDPDGKFVISTRVRCGRSMEGYPFNPCLTEAQYKEMES
3 LIMULUS      PKEWGDINTLVDLDPGGQFIISTRVRCGRSLQGYPFNPCLTAEQYKEMEE
4 APIS         PKDFGDVDSLGNLDPANEFIVSTRVRCGRSLEGYPFNPCLTEAQYKEMEE
5 Consensus     K  GD       DP   F  STRVRCGRS  GYPFNPCLTE QYKEME

1 PACHYGRAPSUS KVFSTLSSLEGELK 164
2 CARCINUS     KVSSTLSNLEGELK
3 LIMULUS      KVSSTLSSMEDELK
4 APIS         KVSSTLSGLEGELK
5 Consensus    KV STLS  E ELK
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Fig. 1: Multiple alignment of *Pachygrapsus marmoratus* arginine kinase partial amino acid sequence (AF288785) with three arthropod sequences: *Carcinus maenas* (AF167313), *Limulus polyphemus* (P51541) and *Apis mellifera* (AAC39040).

Total RNA was isolated from anterior ("respiratory") gill 6 and posterior ("ion transporting") gills 7 and 8. Poly-A mRNA was reverse transcribed to single-stranded cDNA using an oligo-dT primer. To amplify a cDNA fragment coding for arginine kinase, published

degenerate primers (AK2F, AKENDR2; Kotlyar et al., *J. Exp. Biol.* 203: 2395-2404, 2000) were employed. The PCR product was purified and sequenced at MDIBL's Marine DNA Sequencing Center. GenBank analysis revealed a 164-amino-acid fragment showing a very high homology to other published arginine kinases (Fig. 1).

For mRNA expression analysis, total RNA samples from 4 individuals were pooled for each tissue. Identical amounts of template were employed in the subsequent reverse transcription and polymerase chain reactions and PCR product abundance was determined in log-phase so that the strength of the PCR signal was proportional to the initial presence of the target mRNA (Towle et al., *J. Exp. Biol.* 200: 1003-1014, 1997).

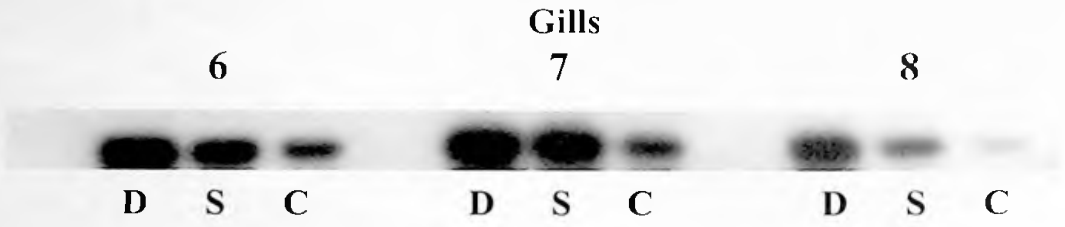

Fig. 2: Expression analysis of arginine kinase mRNA abundance using quantitative RT-PCR. Demonstration of apparent arginine kinase mRNA abundance in total RNA extracted from gills 6, 7 and 8 of *Pachygrapsus marmoratus* acclimated to diluted seawater (D), seawater (S) and concentrated seawater (C).

The quantitative RT-PCR analysis demonstrated a high expression of arginine kinase mRNA in all gills at low salinity (D) (Fig. 2). With the exception of posterior gill 7 of seawater acclimated crabs, gill arginine kinase mRNA abundance increased dramatically with decreasing salinities suggesting a greater transcriptional response of the gene coding for this energy restoring enzyme under challenging low environmental salinities. These results are in contrast to previous findings in the euryhaline portunid crabs *Carcinus maenas* and *Callinectes sapidus* where arginine kinase mRNA abundance was at the same level in anterior and posterior gills and remained constant in crabs acclimated to seawater or diluted seawater of 10‰ and 5‰, respectively (Kotlyar et al., *J. Exp. Biol.* 203: 2395-2404, 2000). Whether the semi-terrestrial nature of the habitat of *P. marmoratus* influences the expression of arginine kinase mRNA requires further studies with other grapsid species.

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